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SPOROGENESIS IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 106

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(WITH PLATES I–IV)

Historical review

There have been published during the past three decades a few accounts of sporogenesis in pteridophytes. Since cytological studies have advanced rapidly in recent years, many views which were once prevalent have been proved to be erroneous, or at least to be capable of a different interpretation, and the authors themselves may have at present entirely different views from those which they held when the accounts were published. It seems worth while, therefore, to make a historical review of some of the work hitherto published.

There is first a paper by HUMPHREY (29) on the study of the spore mother cells of Osmunda, Psilotum, and other forms, in which the main purpose was to prove the presence of extranuclear centrosomes in plants above the bryophytes. His account of the number of chromosomes in Osmunda was taken up by STRASBURGER (46) in his data dealing with the periodic reduction of chromosomes in the life-history of organisms, as one of the instances which prove the constancy of the number of chromosomes in a certain period of the life-cycle.

OSTERHOUT'S (35) studies on Equisetum were really one of the most important contributions to our knowledge of the spore mother cells of higher plants. He found that the spindle at the outset is multipolar polyarch, according to the terminology of STRASBURGER (47), and that the numerous cones become grouped around the common axis, but the poles of the spindle at metaphase hold their composite nature with entire absence of the focal center or centrosome. This account, which did much to dispose of the current idea that the development of the spindle in higher plants is controlled by centrosomes, is noteworthy.

Then appeared two papers with detailed description of the method of division and distribution of chromosomes: one by CALKINS (11) on *Pteris tremula* and *Adiantum cuneatum;* and the other by STEVENS (44) on *Pteris aquilina*, *Cystopteris fragilis*, and *Scolopendrium vulgare.* CALKINS believed that there was a tetrad formation of chromosomes in the prophase of the spore mother cell of ferns, and that the process of sporogenesis in those forms followed the same course as was observed in the maturation division of sexual cells in animals, with a transverse division and consequently a qualitative reduction in WEISMANN'S sense. STEVENS' studies on the forms named above disagree with CALKINS' view in several particulars, the main point being that there is present no transverse division of chromosomes in the spore mother cell, but two longitudinal divisions, and therefore there is merely a quantitative reduction. STEVENS' observation on the formation of daughter chromosomes in the second division was criticized by STRASBURGER (47).

SMITH (42) investigated the sporogenesis of *Osmunda regalis.* His results agree with those of OSTERHOUT in regard to the absence of centrosomes, and differ as to the origin and development of the spindle; he held that the spindle was bipolar from the beginning.

In 1904 GREGORY'S paper (26) was published. His account covers the sporogenesis of eight genera of ferns, i. e., Pteris, Scolopendrium, Asplenium, Onoclea, Davallia, Fadyenia, and two other forms among Cytheaceae, and seems to confirm FARMER and MOORE'S interpretation (18) of the formation of chromosomes at the heterotypic division, indicating the occurrence of a transverse division.

The next year STEVENS (46) published his results on the spore formation of *Botrychium virginianum.* His account begins with his so-called early prophase of the spore mother cell; the first appearance of the spindle of the first and second divisions, he finds, is within the nucleus. As regards the heterotypic mitosis, he states that the chromosomes in metaphase separate in the line of transverse fission and that he agrees with the views maintained by FARMER and MOORE (**18**), GREGORY (**26**), and STRASBURGER (**49**).

CARDIFF'S (**12**) studies of synapsis and reduction in several forms of flowering plants and in *Botrychium obliquum* paid particular attention to the formation of the spirem and its behavior up to the formation of bivalent chromosomes in the heterotypic mitosis. According to him, paired threads, which appear in the presynaptic stage of the nucleus, fuse during synapsis and again split longitudinally after synapsis, probably along the line of the previous fusion.

In 1907 a paper entitled "Studies in apospory and apogamy in ferns" appeared under the joint authorship of FARMER and DIGBY (**16**), whose two preliminary notes were published in 1903 and 1905. This paper deals directly with the matter indicated by the title, but some stages in the division of the spore mother cell are described in three forms of Lastrea and one species of Scolopendrium. The paper is accompanied by many important figures, but only three of the spore mother cells with heterotypic chromosomes are given, so far as sporogenesis is concerned.

Recently there appeared two papers, one by BURLINGAME (**10**) on Ophioglossales, and the other by GRÉGOIRE (**24**), with the title "La formation des gemini hétérotypiques dans les végétaux." BURLINGAME'S paper is chiefly devoted to a study of the sporangium. From the cytological standpoint GRÉGOIRE'S paper is very important. His views, which were published in 1904 (**25**), concerning the structure of the resting nucleus of the spore mother cell, interpretation of synapsis, formation of chromosomes of the heterotypic mitosis, and distribution of the daughter chromosomes in the first and second divisions, were based chiefly upon BERGH'S studies (**6, 7**) of Allium, Lilium, and other forms; but in this recent paper GRÉGOIRE records the results of his own examination of *Osmunda regalis* and four monocotyledons, which confirm his previous views.

Nephrodium

My present investigation of *Nephrodium molle* Desv. was carried on in order to understand the characteristics of the nuclear behavior of this species, and to obtain the knowledge prerequisite for a study of apogamy in the same species. Consequently this paper should be regarded as introductory to a paper entitled "A study of apogamy," which will appear later. Much attention has been paid to the behavior of chromosomes, combining observations with theoretical views, with a hope of securing an explanation of the phenomena observed, so that the chromatin substance will be the chief object treated in the present paper.

I wish to express my gratitude to Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN for their kind advice and criticism during the progress of this work. I am also under obligation to other members of the botanical staff for courtesies extended to me.

MATERIAL AND METHODS

The material for this investigation was collected in greenhouses of the Hull Botanical Laboratory, and in Lincoln and Washington parks, Chicago, from October 1906 to March 1907.

Killing and fixation were made in various fluids, among which FLEMMING'S weak solution, with a little modification in the percentage of acetic acid, proved most effective. The material was left in the fluid for two to four hours, then transferred into fluid of the same formula, but without osmic acid, ten to twenty hours. The thoroughly washed material was passed very carefully through a series of alcohols beginning at three per cent. and imbedded in 48° to 52° paraffin. The sections were cut 3–5 μ in thickness and stained with safranin gentian-violet, or iron-alum-hematoxylin, sometimes combined with counter-stains, such as safranin, orange G, eosin, or Congo red. A Zeiss apochromatic immersion 1.5^{mm}, N. A. 1.30, and compensating oculars were used for studying the preparations.

DESCRIPTION

Vegetative mitosis in the sporophyte

A leaf too young to produce sori shows in its various parts the successive stages in vegetative mitosis. The form of the mitotic figure is affected by irregularities in the size of the cell which contains it, and the abundance of plastids, oil, and other cell contents interferes somewhat with accurate observation. The mitotic figures which occur during the formation of sporogenous tissue, from the first division of the archesporial cell to the completion of spore mother cell, proved most favorable, being entirely free from such obstructions, and these mitoses will be used for the following description.

The resting nucleus in the sporogenous tissue contains a chromatin reticulum with one or several nucleoli. The general structure of the nuclear reticulum needs mentioning. It does not seem to consist of two distinct elements, chromatin and linin, with chromatin granules distributed irregularly upon or imbedded in the linin ground-substance, but it seems to be composed entirely of chromatin material. The appearance which indicates that there may exist a mixture of two substances—the dark-stained masses and light-colored strands—does not seem to be due necessarily to a different affinity for stains, but to the fact but that the more compact regions of chromatin material are more affected by stains than the more loosely aggregated regions, which are lightly stained.

The number of nucleoli is variable and there seems no rule for their position in the nuclear cavity, for sometimes they lie very close to the membrane and sometimes they are centrally located. The form is rounded in most cases, but often they are aggregated into one or two irregular masses. Even in the resting nucleus they show a vacuolized structure.

The chromatin reticulum in the resting nucleus is irregularly distributed, often great masses being located in the center or sometimes eccentric near the periphery, leaving the center vacant. Approaching the prophase, there seems to be a tendency of some of the chromatin material to form a continuous spirem, i. e., some fine strands of the chromatin may break apart entirely or may become thicker by getting the material from some ragged chromatin clumps which consequently diminish in size. The spirem, uneven at first, becomes gradually uniform in thickness and distributed throughout the nuclear cavity (*fig. 1*). Whether there is present a single spirem or not was not determined. Close examination of this stage in a number of preparations showed that it was hardly possible to make out free ends, and this may be taken as an evidence that the spirem is a continuous one. Nucleoli persist in positions distinctly isolated from masses of the chromatin reticulum, so that they do not seem to contribute to the establishment of the spirem through the migration of material. Usually at this stage they seem to unite into one mass.

While these events are going on within the nucleus, there are formed kinoplasmic caps at two poles outside. The kinoplasmic cap at first consists of a few fibers arranged in a cone, the fibers evidently running in a longitudinal line, one end focusing at one pole, and the other end diverging toward the nucleus, reaching nearly to the nuclear membrane or falling short of it. Since no change ever occurs in the nuclear membrane, these fibers certainly originate from the rearrangement of the cytoplasm where the cone arises. The whole cytoplasm, except where the kinoplasmic caps arise, shows at this time a fine fibrillar structure. Now the nuclear membrane begins to disappear at the basal region of the kinoplasmic caps, the rest of it still clearly limiting the nuclear contents from the cytoplasm outside. There seemed to be no regularity in the course of the spirem until this stage. The entangled spirem was distributed throughout the cavity, the bent parts of the loops being directed toward the membrane; but when the nuclear membrane begins to disappear near the kinoplasmic caps, some parts of the spirem seem to move out in the direction of the caps.

Then follows the entire disappearance of the nuclear membrane, the segmentation of the spirem into a certain number of slender chromosomes, and the intrusion of spindle fibers which become attached to the chromosomes at their ends (*fig. 2*). At this time there is first observed the longitudinal splitting of the chromosomes, the two parts resulting from the splitting always remaining closely associated. The chromosomes, in general, are directed along the axis of the two poles; one end reaches near the pole, and about the equatorial region lies the other end, to which spindle fibers from the two opposite poles are attached; so that the distance of the attached ends from the poles is approximately the length of two chromosomes. This stage passes into the condition shown in *fig. 3*, where the chromosomes become much less curved. The line associating the two chromosomes which resulted from the longitudinal splitting is still visible. The chromosomes which were so slender and peculiarly curved, as stated above, from the first segmentation to this period, now begin to shorten; the equatorial ends, to which the spindle fibers from the two poles are attached, remain stationary, while the ends directed to the poles move toward the equator as the chromosomes shorten. In the course of shortening, a stage such as that represented in *fig. 4* has been described by some authors as being a typical figure of vegetative mitosis in ferns.

However, the shortening of the chromosomes proceeds still farther, until the bent ends lying at the equatorial region become straightened, and the chromosomes become rod-shaped; thus there is established the stage in which the chromosomes are arranged in an equatorial plate (*fig. 5*). The two daughter chromosomes formed by the longitudinal splitting of a single one at prophase have remained side by side and have been affected equally by the process of shortening, so that now they are almost similar in size and form. When this stage of the equatorial plate is attained, the two daughter chromosomes seem to begin to separate at once. This stage is less frequently met than the earlier or later stages, even in material favorably fixed and showing an abundance of stages.

The separation of many daughter chromosomes generally begins and proceeds simultaneously, but rather rare instances are observed of several tardy ones, which separate after their associates are already on the way toward the poles. The chromosomes arranged in the equatorial plate are perfectly straight; the separation of the two halves begins at the ends where the spindle fibers are attached and proceeds toward the other end, necessarily causing a curving, as represented in *fig. 6;* but soon after their complete separation they resume their straight form and so persist. When the two sets of daughter chromosomes reach nearly to the poles, they become perfectly parallel, without overlapping (*fig. 8*), and in the more favorable cases they are as regularly placed as if they stood in one plane, a stage which is the most satisfactory for counting. The polar view of this stage (*fig. 9*) shows that the number of chromosomes is 128 or 132. During prophase and even metaphase the number of chromosomes could sometimes be estimated, especially in the polar view at the equatorial plate stage, but not with such exactness as in the early telophase.

The chromosomes grouped at the pole are drawn so tightly together that the ends toward the pole become closer than the ends directed toward the equatorial region. This aggregation process goes on farther and there results an extremely dense mass, in which it is difficult to trace the outlines of each chromosome. The chromosomes begin to be vacuolized, i. e., chromatin materials which constituted the chromosomes in their compact condition become localized in an irregular way, in some places becoming quite loose, and in others still remaining as a dense aggregation. All the chromosomes undergoing this change are associated, the ultimate result being a vacuolized mass of chromatin material from all the chromosomes (*fig. 10*). By the interaction between the cytoplasm which surrounds these vacuolized masses and nuclear sap which may be secreted within the vacuolized region of the chromosomes, there is formed a nuclear membrane. As the nuclear sap increases in amount, the nucleus grows in size (*fig. 11*).

The polar end of the daughter nucleus at the time of the formation of the membrance is always concave (*fig. 12*), very likely due to the fact that before the vacuolization the face ends of daughter chromosomes pointed to the pole are drawn together toward the pole so as to come nearer, and that in this position vacuolization has proceeded. It seems probable that the vacuolization is more pronounced in that concave region than elsewhere, so that in the young daughter nucleus the chromatin material is rather scanty at the region of the previous pole, where immediately after the organization of the daughter nucleus one or two new nucleoli appear (*fig. 13*). It may turn out that the daughter nucleus thus reconstructed shows polarity.

Coincident with the construction of the daughter nuclei there appear granules in an equatorial plane upon fibers of the central spindle. The granules first make their appearance upon the fibers in the central part of the equatorial plane and then toward the periphery. It seems reasonable that the fiber substance has contributed the material for the formation of the granules.

Contemporary with the completion of the cell wall, growth of the daughter nuclei continues and the chromatin material assumes again a ragged structure on account of further continuation of vacuolization. The general outline of the daughter nucleus becomes nearly spherical and there is established the resting nucleus of the succeeding mitosis (*fig. 14*).

The processes of nuclear division from the reticulum of the resting condition to the reconstruction of the next resting nucleus are carried on as described above in any vegetative mitosis in Nephrodium, and the sequence of mitosis preceding the formation of the nucleus of the spore mother cell is exactly the same.

Sporogenesis

PRESYNAPTIC STAGE.—The chromatin reticulum in the resting nucleus of the spore mother cell has the same structure as was just described for the nucleus of the sporogenous cell; the size of the nucleus, after its full growth, is greater than that of any nuclei of the sporogenous cells, and not infrequently it occupies almost all of the cell. No regularity could be found in the massing of the chromatin material; at certain points there are dense masses and at others almost none at all. Nucleoli, one to several in number, are always present in various locations, without any physical connection with the chromatin.

The exact structure of the chromatin reticulum needs description. It is an anastomosing complex of ragged clumps and irregularly branched strands (*fig. 15*); the former always stain deeply and the latter lightly, simply because of the difference in compactness of massing. They originated from a single source—the chromosomes of the previous mitosis—by the vacuolization process described above.

The clumps of the reticulum lose gradually their ragged look, because of the disappearance of the fine strands, the elongation of the clumps in certain directions, and the migration of material from the clumps to some of the fine strands so as to thicken them (*fig. 15a*). All these changes tend to transform the ragged structure, but they do not occur simultaneously throughout the reticulum, so the whole is still plainly a reticulate structure, as seen in *fig. 16*. Nevertheless, in regions where the reticulum is being converted into thread structure there are visible parts of threads running closely side by side (*fig. 16a*).

SYNAPSIS.—The transformation of the reticulum into a thread structure now proceeds rapidly. When the transformation is nearly completed (*fig. 17*), parts of the thread are not smooth and uniform in appearance and thickness, and in its course it is greatly tangled. These tangled masses of thread begin to contract and become localized on one side of the nuclear cavity, a process which marks the initiation of the synaptic state. Preparations stained with iron-alum-hematoxylin and extracted to a certain degree of differentiation of the stains, show that this loosely tangled mass consists of lightly colored threads and deeply stained granules; undoubtedly the thread in this tangled and contracted condition is not homogeneous throughout; on the contrary, there are present not only irregularities in thickness in parts of the thread, but also the chromatin material is more compact in certain parts and more loosely aggregated in other parts.

That the parts of the thread in this stage are double is fairly discernible (*fig. 17a*) in tangential sections of the nucleus; undoubtedly this doubling has come from the changes observed in the earlier stage represented in *fig. 16* as originating from the transformation of the reticulum; therefore this double part of the thread cannot be recognized as an indication of a new longitudinal splitting, but as two threads independent from the start.

These double threads run side by side very closely, as if they were fusing. Whether they are completely fused or not could not be determined, but their association is very close. These masses of threads become more tightly drawn together and contracted into a ball which seems to be the climax of the synaptic stage (*fig. 18*).

FORMATION OF HETEROTYPIC CHROMOSOMES.—The course of the thread or spirem is irregular in the synaptic stage; it is twisted and generally much curled, but there are present several parts of the spirem which run comparatively straight across the contracted ball of the spirem from near the nuclear wall with which it lies in contact. The loops happen to be formed near the periphery of this contracted mass, but they have nothing special to do with the formation of chromosomes.

In the majority of cases the nucleoli seem to unite into one, which becomes caught in the tangled mass in the early synaptic stage, generally far from the nuclear membrane, and its spherical form persists.

The spirem of this stage shows a relatively smooth structure and it is not easy to distinguish its double nature throughout, probably on account of the close association of the two independent parts; but tangential sections reveal that the parts of the spirem are unmistakably composed of two threads running side by side, often diverging rather widely (*fig. 19*). It seems highly probable that we see here an indication of the separation of two threads which were first observed in the presynaptic stage (*figs. 16, 16a*) and entered into synapsis, as already described. Now the tangled mass of the spirem begins to uncoil (*fig. 20*) slowly; superficial parts of the spirem in the synaptic mass at first become widely distributed, while the unraveling of the inner parts of it are naturally delayed, and the longitudinal separation of the two independent parts is observable in the portions of the spirem which are widely uncoiled (*fig. 20*).

The spirem becomes considerably thickened, certainly due to shortening, and becomes distributed loosely through the cavity; while the line of the longitudinal splitting or separation of the spirem into two elements seems obliterated, but its presence is evident in some places (*fig. 21*).

The course of the spirem again becomes irregular, and in some parts there are present complicated tangled masses of the spirem on account of the twisting of the spirem itself, but double threads, the constituents of the spirem, run mostly in close association (*fig. 22*). Shortening and thickening of the spirem proceeds until it segments into chromosomes. As shown in *figs. 23a* and *23b*, the segmentation is not simultaneous throughout the entire spirem, but finally there are formed 64 or 66 chromosomes, each bivalent in nature (*figs. 24a, b, c*).

The two halves of the bivalent chromosomes lose their close association after their segmentation, and show every possible position and connection with each other, such as H, I, J, K, L, O, T, U, V, X, Y, and so forth, and even give an impression of being of different length and size, mostly due to the point of view. The chromatin material which constitutes the surface region of the chromosomes seems loosely aggregated, but it is compactly massed in the center, and there are observed faintly stained substances of fibrillar form attached at the ends of chromosomes, which seem to be remnants of the material of the spirem left over at the time of segmentation, probably on account of the viscid nature of the spirem. The loosely aggregated chromatin material is gradually drawn and condensed toward the central part, but the condensation process takes place irregularly, so that for a while the chromosomes appear spiny. The chromosomes become shortened, and the loose fibrillar appendages are also shortened by contributing material to the main body of the chromosome, yet some can still be seen (*figs. 25a, b, c*).

A short description of the chromatic substance seems necessary. Early in the resting stage of the nucleus, the cytoplasm shows a fine fibrillar structure, almost homogeneously distributed. When the nuclear structure begins to enter into synapsis, long kinoplasmic fibers appear from the rearrangment of the cytoplasm surrounding the nucleus, but far apart from the nuclear membrane. Between these kinoplasmic fibers and the nuclear membrane there is present a clear region free from any fine fibrillar structure, which was the main feature of the other parts of the cytoplasm before the appearance of the kinoplasm (*fig. 21*). Some of the stages showing such a process are represented in *figs. 21–25*. The nuclear membrane, which has stained distinctly, now becomes less and less affected by stains and seems to be dissolved entirely, but for a short time the encroaching and anastomosing kinoplasmic fibers preserve the previous nuclear outline (*figs. 25a, b, c*). The kinoplasm, as a rule, surrounds the nuclear membrane, but some of the threads run in somewhat radial directions. No distinct polar formation was observed.

When the kinoplasmic fibers push into the nuclear cavity, some of them are seen to be attached to the chromosomes, while others pass through, and a multipolar structure becomes noticeable (*figs. 26a, b*); from the fibers of the multipolar spindle there is worked out the bipolar spindle, as indicated in *figs. 27a, b*. Shortening of the chromosomes continues always up to this time, so that now there result very short bivalent chromosomes, different in aspect from those in the early prophase. There is no regularity in the position of the chromosomes, which are found on almost all parts of the spindle, yet it is not hard to notice from successive stages, as *figs. 26a, b* and *27a, b*, that there is a tendency to move toward an equatorial region until 64 or 66 chromosomes are arranged in the equatorial plate.

The two parts of the bivalent chromosome in the equatorial plate are applied to each other throughout their entire length; ends in which spindle fibers are attached become directed toward the poles, while the other ends remain in contact. The polar view of the equatorial plate or a little earlier stage is favorable for counting the chromosomes, which showed always 64 or 66, each bivalent in nature (*fig. 29*).

SEPARATION OF THE DAUGHTER CHROMOSOMES.—Now the daughter chromosomes begin to separate; some of them, on the way toward the pole, show a V-shape, the angle being directed toward the pole. This does not seem to have resulted from the bending of one daughter chromosome, because it always remains straight after separation; hence the V-shape may be regarded as resulting from a new longitudinal fission of the daughter chromosome, providing for the two granddaughter chromosomes of the second division. This longitudinal fission occurs only at the end of the chromosome directed toward the equatorial region, without proceeding to the other end. The two arms of the V come into contact again as the daughter chromosome reaches the pole.

As a rule, the sets of daughter chromosomes pass to the poles simultaneously, but there were observed some exceptional cases where some were much delayed (*figs. 31, 32*); and rarely the irregularity is so extreme that tardy chromosomes are left behind, while their associates are already in an aggregated condition at the pole (*fig. 34*).

RECONSTRUCTION OF THE DAUGHTER NUCLEI.—When the group of daughter chromosomes reaches the pole, each chromosome for a short time remains fairly straight, which is favorable for counting the chromosomes from the polar view (*fig. 35*). Then the vacuolization process begins (*fig. 36*). There is a certain point of difference in detail between this process and that described in the reconstruction of daughter nuclei in vegetative mitosis; the polar ends of the daughter chromosomes do not seem to be drawn together so as to give a concave form, but begin to be vacuolized at once, while in a state of loose association. The vacuolization is prominent along the line of the longitudinal fission of the daughter chromosomes, a line which became obliterated when the chromosomes reached the pole. After the formation of the nuclear membrane it is not impossible to recognize a certain part of the chromatin substance as representing a certain chromosome (*fig. 38*); in other words, the vacuolization process in the telophase of the heterotypic mitosis does not cause the chromosomes to return into the ragged chromatin reticulum from which they are formed, but the reconstruction stops before the individual outline of the main body of the chromosome is lost entirely. Therefore, the daughter nucleus does not pass into the resting stage with ragged chromatin reticulum in the telophase of the first division, but always shows the main body of individual chromosomes recognized in V-shape, notwithstanding the nucleus grows considerably in size after the formation of the membrane. No nucleolus was observed in the daughter nucleus (*figs. 40, 41*). The nucleus in such a condition is ready for the second mitosis.

GRANULAR ZONE DIVIDING THE SPORE MOTHER CELL.—During the nuclear division described above, the cytoplasm generally has a fine fibrillar structure, with vacuoles here and there near the cell wall. When the chromosomes are arranged at the equatorial plate, fibers running from the poles cross in the equatorial region near the cell wall, where the cytoplasm begins to be transformed into the granules of this stage. After the organization of the daughter nucleus, the granules begin to increase and proceed rapidly toward the center along an equatorial plane of uniform thickness (*fig. 38*), at first avoiding only the space which was occupied by the central spindle (*fig. 39*). At last even the middle parts of the central spindle become replaced with or transformed into granules, so that finally the protoplast of the spore mother cell is entirely separated by a zone of these granules into two hemispheres, each with its daughter nucleus. The greater portion of the central spindle, which contributes to the formation of the granules, returns into the fibrillar structure of the cytoplasm at telophase.

CHROMOSOMES OF HOMOTYPIC DIVISION.—As described above, the daughter chromosomes which entered into the reconstruction of the daughter nuclei do not lose their individuality, but it is possible to recognize their identity even after they are combined and anastomosed together into a reticular structure (*fig. 41*). The recognizable V elements in the nucleus of the second division are what undoubtedly resulted from the influence of the vacuolization on the line of the longitudinal fission of the daughter chromosomes in the first mitosis during the last telophase; however, the arrangement of V's which they had when vacuolization began at the last telophase does not seem to have been maintained, for the closed angles of the V's are now visible on both upper and lower sides of the nucleus from such a side view as shown in *fig. 42*. Such a change in the situation of V's during the course of the vacuolization process and nuclear growth might easily occur because of the bending and curving due to their being gathered into the round cavity of the daughter nucleus.

As the disappearance of the nuclear membrane approaches, V elements become more distinctly pronounced (*fig. 43*), some of the fine strands which branched out from V's disappear, but irregularity in thickness persists and the arms of the V's end in very slender threads. The general outline of the nucleus up to this time is always flattened, so that the nucleus shows two different aspects according to whether the view is from the pole or equator of the spore mother cell; hence the same mitotic figure of this nucleus, no matter what stage it may be, will always present two different side views, one being always narrower than the other.

As the nuclear membrane breaks down, the cytoplasmic fibers enter into the nuclear cavity, where the chromosomes are crowded (*fig. 44*). The figure of this stage recalls the combined features of both the heterotypic and the typical forms: the chromosomes, long, slender, and curved, with daughter chromosomes in pairs, resemble those of the vegetative mitosis; the achromatic fibers do not construct a cap, but appear multipolar as in the heterotypic case, although they are from the start observed near the nuclear membrane instead of at a distance from it. The two arms of the V's, which now provide for the two daughter chromosomes of this division, are still hanging together at the angle (*fig. 44*) at which spindle fibers seem to become attached. When the multipolar condition has passed into the bipolar spindle, the angle ends of the V's, or the ends where spindle fibers are attached, occupy the position near the pole, while the other ends diverge toward the equatorial region (*figs. 45, 46*). The stage resembles that represented in *fig. 3* as one of the transient conditions in passing to the stage of the equatorial plate.

Gradually the divergent arms of the V's, or the daughter pair of the bivalent chromosomes in this division, draw near to each other until finally they come into close contact. Simultaneously, the bivalent chromosomes, each consisting of two daughter chromosomes, move toward the equatorial region; as the ultimate result, the ends of chromosomes to which fibers are attached are arranged in an equatorial plane, while the other free ends are directed toward the pole (*fig. 47*). The figure of this stage is also comparable with that of the vegetative mitosis shown in *fig. 4*. The shortening and thickening of the bivalent chromosomes begin and proceed in similar rate and degree in each member of the pairs, which is always in contact with its fellow (*figs. 48, 49*). These processes are exactly similar to those which have already been described in the vegetative mitosis. The bivalent chromosomes are finally shortened into straight, rod-shaped chromosomes and become arranged in an equatorial plate (*fig. 50*). Some of the bivalent chromosomes in this stage of the second division, unlike that of the first division, are slightly above or below the plane of the rest, so that the polar view is not satisfactory for accurate counting (*fig. 51*).

The separation of the daughter chromosomes which constitute the bivalent chromosomes begins at once. Generally daughter chromosomes pass in a group toward the pole, but occasionally the separation of the two halves of certain chromosomes is more delayed than the rest (*figs. 52, 53*). In any event, the daughter chromosomes which are on the way toward the pole have a straight rod shape, a little more elongated than they were when arranged in the equatorial plate. When the daughter chromosomes reach the pole they keep for a while a regular and almost parallel arrangement (*figs. 54, 55*), which was advantageous for counting. The number is 64 or 66 (*fig. 56*).

The group of chromosomes becomes more and more closely aggregated (*figs. 58, 59*). When the vacuolization begins, the individual outlines of the chromosomes are gradually lost, and after the organization of the nuclear membrane, which is formed as in the vegetative mitosis, the growth of the nucleus follows (*fig. 60*).

Simultaneous with the reconstruction of granddaughter nuclei the cell plate is laid down between them, exactly as in the vegetative mitosis (*figs. 57–60*). The second division forming two granddaughter nuclei takes place in the daughter nuclei of both hemispheres, separated already by the granular zone in the first mitosis. From the cytoplasm which filled the space between two granddaughter nuclei new spindle fibers now arise connecting them. These fibers necessarily pass the granular zone, in the middle plane of which is established the cell plate that really divides the two daughter protoplasts of the heterotypic mitosis (*fig. 61*). As a very rare case, the granular zone is not in a plane, and the fibers which connect the granddaughter nuclei of different hemispheres arise before cell plates are laid down between them, so that the protoplast of the spore mother cell in this case becomes divided simultaneously into four tetrahedral spores.

Discussion of cytological phenomena

NUCLEAR RETICULUM.—Since the terms "chromatin" and "linin" were given by FLEMMING (21) and by SCHWARZ (42) respectively to the substance of the nuclear network which stains deeply and to the substance of the achromatic reticulum, these two substances have been regarded as essential constituents of the nucleus in its resting stage, and it became the general formula in describing the resting nucleus to state that it contains a fine network of linin fibers in which are imbedded chromatin granules.

To determine the real nature of chromatin and linin, and to find out the origin and differentiation of these two elements needs special investigation, which is not, of course, the object of the present work; however, the observation of events that occur between the telophase of one mitosis and the prophase of the next gave the writer the following notion regarding the nature of chromatin and linin, similar to the conclusion drawn by GRÉGOIRE and WYGAERTS (25).

When a group of daughter chromosomes reaches the pole, they are first drawn tightly together, so as to lose, apparently, their individual outlines. Vacuolization then takes place in this mass, and with the appearance of vacuoles parts of the mass of chromatin substance become irregularly separated and distributed so as to become transformed into a ragged reticular structure consisting of anastomosing, irregular clumps and finer, branched strands. The membrane, which may be formed by the contact of the surrounding cytoplasm with the nuclear sap that appears in the vacuoles, separates the mass of chromatin substance from the cytoplasm outside at a very early period, so that it is highly probable that no achromatic structures are included within the nucleus. The nuclear reticulum of the resting stage is nothing but the result of further advancement of the vacuolization. The new spirem of the ensuing division is formed again by the rearrangement of the ragged chromatin reticulum.

From the foregoing it seems quite probable that the nuclear reticulum is derived entirely from the chromosomes, the lightly staining threadlike strands in the resting nucleus being only a part of the chromatin substance that has assumed such a structure.

REDUCTION OF CHROMOSOMES AND SYNAPSIS.—Since tetrads were first figured by HENKING (28) in the maturation division of an insect, several authors have reported the tetrad formation in various animals in connection with the reduction of chromosomes. From studies of the plant cell, the presence of the tetrads in the spore mother cell was independently described by OSTERHOUT (35) and CALKINS (11). OSTERHOUT figured the tetrads in the prophase of sporogenesis in Equisetum. CALKINS found in Pteris and Adiantum that the tetrads arise from rings or double rods, parallel or crossed, which resulted from the longitudinal splitting of primary chromatin rods or segments and which break up into four parts (the tetrads), followed by a transverse division. An essentially similar mode of tetrad formation was described by BELAJEFF (5) in Iris and by ATKINSON (4) in Arisaema and Trillium.

As already described, there is no tetrad formation in Nephrodium, chiefly due to the fact that both first and second divisions do not occur in rapid succession, and that the appearance of the fission providing for the second division is delayed until after the separation of the bivalent chromosomes of the first mitosis. At present most investigators believe that tetrads, in a strict sense, are not formed in the majority of cases, excepting in a few groups of animals.

The nature of these two successive divisions of chromosomes in the spore mother cell has been discussed by many authors, chief attention being paid to phases after the segmentation of the spirem into a certain number of chromosomes. Among early observers there has been every possible variation of opinion. Miss SARGANT (39) found in Lilium that both divisions were longitudinal. This view was soon concurred in by STRASBURGER (47), FARMER and MOORE (17), and others. Two years later STRASBURGER (50) and MOTTIER (33) concluded that the fission of the chromosomes in the second division was transverse, and essentially similar results were obtained by ISHIKAWA (30) in Allium, and by BELAJEFF (5) in Iris. DIXON (13,14,15) and SCHAFFNER (40, 41) held a different view, believing that the continuous thick spirem twists up into loops that are the inception of chromosomes, and that the chromosomes of the first division result from the loops, separating by a transverse division at the angle of the loops; while the second division is longitudinal. ATKINSON (4) found two different methods in Arisaema and in Trillium; in the former species the first division was transverse, as DIXON and SCHAFFNER found; while in the latter the second was transverse, as STRASBURGER and MOTTIER claimed.

Among these three divergent views, which claim respectively that there occur two longitudinal divisions, that the first division is transverse and the second longitudinal, and that the first division is longitudinal but the second is transverse, the third was of short duration. The authors (STRASBURGER and MOTTIER) who described the existence of such a case changed their opinion and returned to their former view that both divisions are longitudinal, so that there persist only two views, one claiming the presence of two longitudinal divisions, and the other claiming that the first is transverse: the former one of these was held by GUIGNARD (27), GRÉGOIRE (22), LLOYD (31), and others.

However, the more recent studies of synapsis have involved very detailed and accurate observations concerning the nature of the reduction division. Some of the investigators mentioned above have changed their views, while others still maintain them, but in both cases a new or modified notion concerning the origin of chromosomes and the nature of reduction division is held.

As described before, the nucleus of Nephrodium in the presynaptic stage consists of a complex, anastomosing ragged chromatin reticulum. This ragged reticulum shows a tendency to become transformed into a thread structure, but the process does not occur simultaneously in different regions. When the transformation has taken place, the two parts of the thread are observed running side by side from the first. Such a condition as is represented in *figs. 16* and *16a* is evidently what was called "leptonema" by WINIWARTER (51). Further transformation of the thread structure from the ragged reticulum results in a nucleus with a continuous chromatin thread or spirem whose double nature is only visible at certain parts on account of the close association. The thread becomes tangled and contracted in one side of the nuclear cavity (*figs. 17* and *17a*) until finally there results the climax of the synaptic stage (*figs. 18, 19*); these two stages may cover the "zygonema" condition of WINIWARTER.

The above brings the writer to the conclusion that the spirem is of double nature from the outset, probably derived from the paternal and maternal source, as was interpreted by ALLEN (**1, 2, 3**), GRÉGOIRE (**25**), BERGH (**6, 7, 8, 9**), ROSENBERG (**37, 38**), and CARDIFF (**12**).

In preparations stained with iron-alum-hematoxylin and extracted so as to obtain a proper differentiation, the paired chromatin threads of the spirem appear as if consisting of darkly stained granular parts and a lightly colored ground-substance of threadlike structure, but this simply means that they are not of homogeneous thickness; the more aggregated the chromatin substance, the more dark appears the stain, and therefore probably the individuality of the two halves of the spirem may not be lost entirely during the close association.

There are observed in Nephrodium a number of parts of the spirem running through the contracted mass from the nuclear membrane where the mass lies in contact (*fig. 18*). GRÉGOIRE (**24**) found that a similar condition of the spirem exists in Osmunda, and that in a later stage the chromosomes resulting from the thick spirem held a similar orientation in regard to the nuclear membrane. From this he concluded that there is no continuous thick spirem in the prophase of heterotypic division of Osmunda. Since in Nephrodium the thick spirem as it emerges from synapsis is always in contact with the nuclear membrane up to the segmentation of chromosomes, the condition shown in *fig. 18*, where the number of parts of the spirem run out from one side of the membrane, may have some significance which we are unable to interpret at present.

The thick spirem emerging from synapsis showed first, at the regions widely uncoiled from the contracted mass, longitudinal fissions which came undoubtedly from the separation of the two independent threads that entered into association during synapsis. The thick spirem simply segments into 64 or 66 bivalent chromosomes, each of the pairs being derived from the independent thread. In a certain stage before the "strepsinema" condition, appearances of looping were also observed in connection with the contracted region, but they had nothing to do with the formation of chromosomes as described by FARMER and MOORE (17, 18, 19), GREGORY (26), FARMER and SHOVE (20), MOTTIER (34), and SCHAFFNER (41).

Consequently, the reduced number of chromosome segments of the heterotypic mitosis in Nephrodium are not two sporophytic chromosomes joined end to end, which come to lie side by side by the approximation of the arms of the loops and break apart at the head of the structure; but they are bivalent chromosomes or pairs of sporophytic chromosomes derived from the two threads of the synaptic stage that are always in close association side by side from the beginning, as claimed by ALLEN, BERGHS, and GRÉGOIRE.

Results obtained by MIYAKE (32) in a number of monocotyledons and by OVERTON (36) in a number of dicotyledons accord in general with this description of the mode of formation and separation of heterotypic chromosomes.

Although the present account of Nephrodium differs widely from the accounts of FARMER and MOORE, GREGORY, STEVENS, and STRASBURGER in the interpretation of the synapsis and the prophase of heterotypic mitosis, yet it agrees in one fundamental point, namely, that the first division is heterotypic and separates whole sporophytic chromosomes.

HOMOTYPIC DIVISION.—That the chromosomes in the second division in Nephrodium divide longitudinally is in accordance with the vegetative mitosis; however, there exists an essential point of difference between the two cases, namely, in vegetative mitosis the resting stage is always intercalated between the chromosomes in the last telophase and those of prophase of the ensuing division, so that each mitosis is followed by a resting stage, and a line of new longitudinal fission of the chromosomes arises in the early prophase of each division; while in the homotypic mitosis no resting nucleus is formed in telophase of the preceding mitosis, and therefore the homotypic mitosis should not be regarded as a complete one, standing by itself. Really an early part of the prophase of the second division may be regarded as being initiated at the first division, because the homotypic mitosis separates two daughter halves of a single sporophytic chromosome of the first division; the partial longitudinal fission of this sporophytic chromosome was begun during the anaphase of the first mitosis, although the fission was obliterated in the last telophase.

According to the count of chromosomes which was made in the telophase of the first division, the daughter nucleus may contain 64 or 66 chromosomes, the reduced number; however, it should be remembered that this is certainly due to the obliteration of the longitudinal fission which appeared in the anaphase, and since this early part of the event of the second mitosis is actually initiated in the anaphase of the first division, the daughter chromosomes should well be considered as bivalent, consisting of two granddaughter chromosomes, although the actual separation is completed in the prophase of the second division. Therefore the homotypic division, in spite of its dependence upon the first division, should be regarded rather as a real part of the reduction division, which completes the process initiated in the heterotypic mitosis; in other words, the reduction division is initiated in the heterotypic mitosis, but the reduction of the first division is only apparent; the real reduction in the number of chromosomes is completed in the telophase of the second division. It follows that the first nucleus which contains the reduced number of chromosomes is the spore, and consequently the spore marks the starting-point of the new generation, as discussed by GRÉGOIRE (25).

CENTROSOME.—The presence of the centrosome among the pteridophytes was reported in the spore mother cell by HUMPHREY (29) in Psilotum and Osmunda, and by CALKINS (11) in Adiantum and Pteris. OSTERHOUT (35) denied the presence of the centrosome in the spindle formation of Equisetum.

In Nephrodium, the spindle always arises from the rearrangement of cytoplasm. In sporogenesis a multipolar polyarch spindle is first observed when the nuclear membrane disappears, while in typical mitosis in the vegetative cell, two kinoplasmic caps first appear at the poles of the nucleus before the disappearance of the nuclear membrane. In both cases when chromosomes are arranged in the equatorial plate, spindle fibers gather into a focal center which lacks the centrosome or centrosphere, and the gathering of the fibers into the focal center persists until the last anaphase or early telophase; however, in no stage of mitosis are there present centrosomes, radiations, centrospheres, or any kind of structure which was believed to control nuclear division. As regards the mechanism of mitosis, the writer is not prepared to enter into any discussion. The centrosome question in connection with the blepharoplast in pteridophytes will be discussed in the next paper, which will deal with gametogenesis and fertilization in Nephrodium.

UNIFORMITY IN THE NUMBER OF CHROMOSOMES.—STRASBURGER'S paper (**46**) entitled "The periodic reduction of the number of the chromosomes in the life-history of living organisms," which was the first enunciation of the significance of sporogenesis and the reduction phenomena in relation to the alternation of generations in plants, was based upon the discovery that there is a uniformity in the number of chromosomes in certain phases of the life-history of the individual. Evidence seems to be accumulating from different groups of plants confirming the notion of constancy in the number of chromosomes in individuals under normal development, although there are instances where different authors report different counting.

Generally speaking, accuracy in counting the chromosomes depends upon the number and form of the chromosomes in the individual under consideration. When the number of chromosomes is large and their form is slender and curved, accurate counting is naturally difficult. In Nephrodium the writer found this difficulty, due to the great number and curved form of the chromosomes, but it was not impossible to make reliable counting by using certain stages in which the chromosomes assumed a straight rod shape and a regular arrangement, characteristic of this species. In sporogenesis there are three conditions favorable for counting. First the prophase after the segmentation of the spirem into chromosomes, up to the stage just before the disappearance of the nuclear membrane, was favorable for counting It might well be expected that two parts of the same chromosome which happened to be cut might appear in two successive sections of the same nucleus, and this would make the counted number larger than the actual. Nevertheless, after carefully examining the mitosis in this stage, it was possible to find out the sections with the least number of cut chromosomes, sometimes only two, as was given in *figs. 24a, b, c* and *25a, b, c, d.* The second condition good for accurate counting is the polar view in the early metaphase; and the third case is the polar view at a certain stage of the late anaphase, in which the chromosomes are arranged near the pole.

In the typical mitosis of the vegetative cell, on the other hand, the prophase previous to the disappearance of the membrane is not good for counting, because of the curved and twisted nature of the chromosomes. The polar view of the late prophase or early metaphase is favorable for gross estimation, but in a certain critical stage of late anaphase, when the chromosomes are in irregular arrangement at the pole, accurate counting is always possible.

From the counting made in the stages mentioned above, the writer believes that there is a constant number of chromosomes in Nephrodium, i. e., 128 or 132 in typical mitosis and 64 or 66 in the reduction division; the result is evidently not in harmony with the conclusion drawn by FARMER and DIGBY (**16**) in their recent paper.

The reason for the presence of two sets of numbers of chromosomes (such as 64 or 66) has not been determined.

Conclusion

Since this study of sporogenesis was carried through as preparatory for the study of apogamy in Nephrodium, the general conclusions will appear in the final paper. Two points, however, may be summarized here: (1) there is uniformity in the number of chromosomes in the sporophyte; and (2) in the normal life-history there is a reduction of chromosomes in sporogenesis.

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EXPLANATION OF PLATES I–IV

All figures were drawn with the aid of an Abbé camera lucida, using Zeiss's apochromatic objective 1.5^{mm}, N. A. 1.30, combined with compensating ocular 18, except *figs. 15a, 16a, 17a,* and *19,* which were drawn with same combination of objective and ocular under higher magnification obtained when the tube was extended to the farthest point.

The plates are reduced one-half the original size, except *plate 1,* which is reduced two-fifths.

PLATE I

Vegetative mitosis in the sporogenous cell in the eight-cell stage

FIG. 1. The prophase; the spirem before segmentation into chromosomes; a kinoplasmic cap with a few fibers at each pole; the membrane beginning to disappear at those points.

FIG. 2. Spirem segmented into chromosomes; chromosomes long and curved, split longitudinally, the two halves running closely in contact.

FIG. 3. Chromosomes arranged parallel along the axis passing from pole to pole, one end of chromosomes in which spindle fibers are attached lying in an equatorial plate.

FIG. 4. The intermediate stage in passing from the previous condition to the stage of an equatorial plate; the chromosomes much shortened, one end where spindle fibers are attached being bent, while the end directed to the pole is straight.

FIG. 5. Metaphase, showing an equatorial plate; chromosomes short and rod-shaped.

FIG. 6. Late metaphase; the daughter chromosomes beginning to separate.

FIG. 7. Anaphase; two sets of the daughter chromosomes on the way toward pole; chromosomes almost straight, rod-shaped, slightly more elongated than metaphase.

FIG. 8. Late anaphase; rod-shaped chromosomes parallel at the pole.

FIG. 9. Polar view of stage given in previous figure, showing 128 chromosomes.

FIG. 10. Group of daughter chromosomes vacuolized, the upper side, for merly the pole, being concave.

FIG. 11. Polar view of stage shown in previous figure; arrangment of chromatin material resulting from vacuolization of chromosomes presents regularity.

FIG. 12. Daughter nuclei after formation of membrane, still of flattened form; cell plate has appeared in middle region of central spindles.

FIG. 13. Slightly oblique side view of daughter nucleus after formation of daughter cell; size increased; two nucleoli have appeared in the previously polar region.

FIG. 14. Polar view of daughter nucleus in later stage than that shown in previous figure; nucleoli apart from chromatin material.

PLATE II

Sporogenesis

FIG. 15. One of three sections of a resting nucleus in spore mother cell; chromatin reticulum without any regularity, looking ragged; three nucleoli are present in this section, some of them vacuolate.

FIG. 15*a*. Portion of chromatin reticulum of previous figure under higher magnification; reticulum consists of irregular clumps and slender strands, giving ragged aspect.

FIG. 16. One of three sections of a nucleus in which is shown the tendency to form the thread structure from a rearrangement of the chromatin reticulum; a single nucleus is seen in this section.

FIG. 16*a*. Portion of chromatin reticulum from *fig. 16* under higher magnification; by the disappearance of some of the strands and elongation of ragged clumps, thread structure is formed; these threads from the outset independent.

FIG. 17. Early synaptic stage; chromatin reticulum, almost transformed into continuous thread, begins to contract at one side of the nuclear cavity.

FIG. 17*a*. Portion of the parallel threads in *fig. 17* under higher magnification.

FIG. 18. Synapsis; nucleolus caught in the contracted mass of thread at a position not in contact with the nuclear membrane.

FIG. 19. Portion of the separating threads from a tangential section, the next to that shown in *fig. 18*, under higher magnification.

FIG. 20. The nucleus emerging from synapsis; uncoiled parts of the spirem show the longitudinal splitting or separation of two threads which entered into close association in synapsis.

FIG. 21. Spirem shortened, thickened, and more uncoiled; line of longitudinal splitting or separation visible; kinoplasmic fibers present surrounding the nucleus, circumscribing the comparatively clear zone near the membrane.

FIG. 22. Spirem more thickened and distribution more irregular than in the preceding stage shown in *fig. 21;* kinoplasmic fibers approaching nearer to the membrane.

FIGS. 23*a*, 23*b*. Two sections of the same nucleus when chromosomes begin to be segmented; two halves of the spirem almost equally thickened and in close association; spirem irregularly contracted.

FIGS. 24*a*, 24*b*, 24*c*. Three sections of the same nucleus after segmentation of spirem into 66 bivalent chromosomes; association of two halves of each chromosome has become somewhat loose, so that various combinations of the two daughter halves are shown, such as H, I, J, K, L, O, U, V, X, Y, and so forth; thin strands of chromatin material visible attached to the ends of chromosomes; two chromosomes (*a* and *b*) are cut into two, so that the two parts of the each chromosome appear in two successive sections.

FIGS. 25*a*, 25*b*, 25*c*. Four sections of the same nucleus; chromosomes show spiny structure; nuclear membrane has disappeared, the outline of nucleus being maintained by the encroaching and anastomosing of fibers; two chromosomes (*a* and *b*) are cut into two, so that the two parts of each chromosome appear in two successive sections.

PLATE III

Sporogenesis continued

FIGS. 26*a*, 26*b*. Two sections of the same nucleus, after intrusion of fibers; multipolar spindle is established.

FIGS. 27*a*, 27*b*. Two sections of the same nucleus, after the multipolar spindle has passed into the bipolar condition; bivalent chromosomes, two daughter halves always in pairs, have shortened considerably.

FIG. 28. Early metaphase, the bivalent chromosomes being regularly arranged in an equatorial plate.

FIG. 29. Polar view of early metaphase; lightly shaded chromosomes represent those which could be seen at a deeper focus; 64 bivalent chromosomes are present.

FIG. 30. Metaphase; daughter chromosomes begin to separate.

FIG. 31. A later stage than that shown in *fig. 30;* some of the daughter chromosomes which lie on the way to the pole indicate a new fission that provides for the second mitosis.

FIG. 32. Anaphase; rate of movement of daughter chromosomes toward the pole is variable.

FIG. 33. Late anaphase; some of the daughter chromosomes at the pole still show V-shape, due to the fission first indicated in *fig. 31*.

FIG. 34. Early telophase; daughter chromosomes are grouped at the pole, two of them being tardy; no V-shaped chromosome present.

FIG. 35. Polar view of stage shown in *fig. 34*, showing 64 chromosomes.

FIG. 36. Telophase viewed from the pole; chromosomes vacuolized; nuclear membrane constructed.

FIG. 37. Polar view of daughter nucleus in a later stage of telophase; vacuolization farther advanced.

FIG. 38. Daughter nucleus begins to grow in size; masses of the chromatin reticulum may represent main bodies of vacuolized chromosomes; granular structure begins to arise along periphery of equatorial region where fibers from poles meet.

PLATE IV

Sporogenesis continued

FIG. 39. Polar view of stage shown in *fig. 38;* granular zone surrounding cross-sections of central spindles.

FIG. 40. Spore mother cell separated into two hemispheres by granular zone; daughter nucleus has grown in size; V elements distributed in chromatin reticulum represent main bodies of vacuolized chromosomes.

FIG. 41. Polar view of daughter nucleus shown in *fig. 40.*

FIG. 42. Side view of nucleus of second division; a little later stage than that given in *fig. 41.*

FIG. 43. Nucleus in same stage as previous figure; only parts of chromatin reticulum visible in one focus are drawn; V elements distinct.

FIG. 44. Prophase of nucleus of second division after disappearance of nuclear membrane; spindle multipolar.

FIGS. 45, 46. Prophase after bipolar spindle is established; these two figures are in the same stage, the former being the view from the pole of the spore mother cell, the latter the view from the equator of the same.

FIG. 47. Divergent arms of V's, i. e., daughter halves of chromosomes of second division have become parallel, the ends where spindle fibers are attached lying in the equatorial region.

FIGS. 48, 49. Late prophase; the two figures represent the same stage, viewed from the equator and pole of the spore mother cell respectively.

FIG. 50. Metaphase; two daughter halves in each bivalent chromosome exactly similar in size and shape.

FIG. 51. Polar view of stage intermediate between the two conditions shown in *figs. 48* and *50*, this aspect being due to the overlapping of the chromosomes.

FIGS. 52, 53. Anaphase; two figures represent the same conditions viewed from the equator and pole of the spore mother cell respectively; two or three pairs of daughter chromosomes are tardy in separation.

FIGS. 54, 55. Late anaphase; the two figures show the same stage viewed from the equator and pole of the spore mother cell respectively.

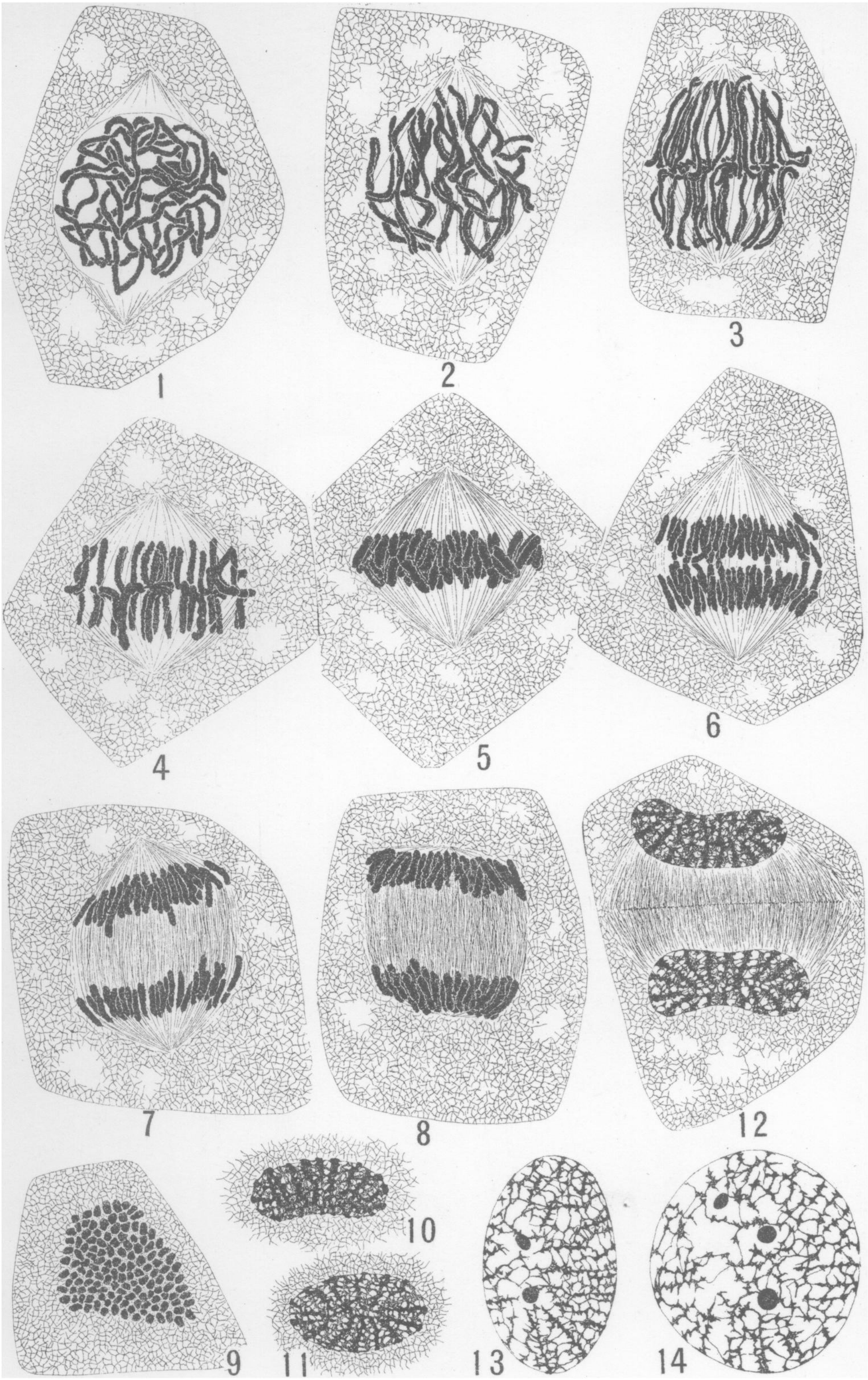
FIG. 56. Polar view of daughter chromosomes of late anaphase shown in *figs. 54* and *55;* 64 chromosomes.

FIG. 57. Early telophase; daughter chromosomes of second division at the pole and arranged regularly; cell plate laid down at middle region of central spindles.

FIGS. 58, 59. Telophase; the two figures show the same stage, viewed from equator and pole of spore mother cell; cell plate has divided the protoplast.

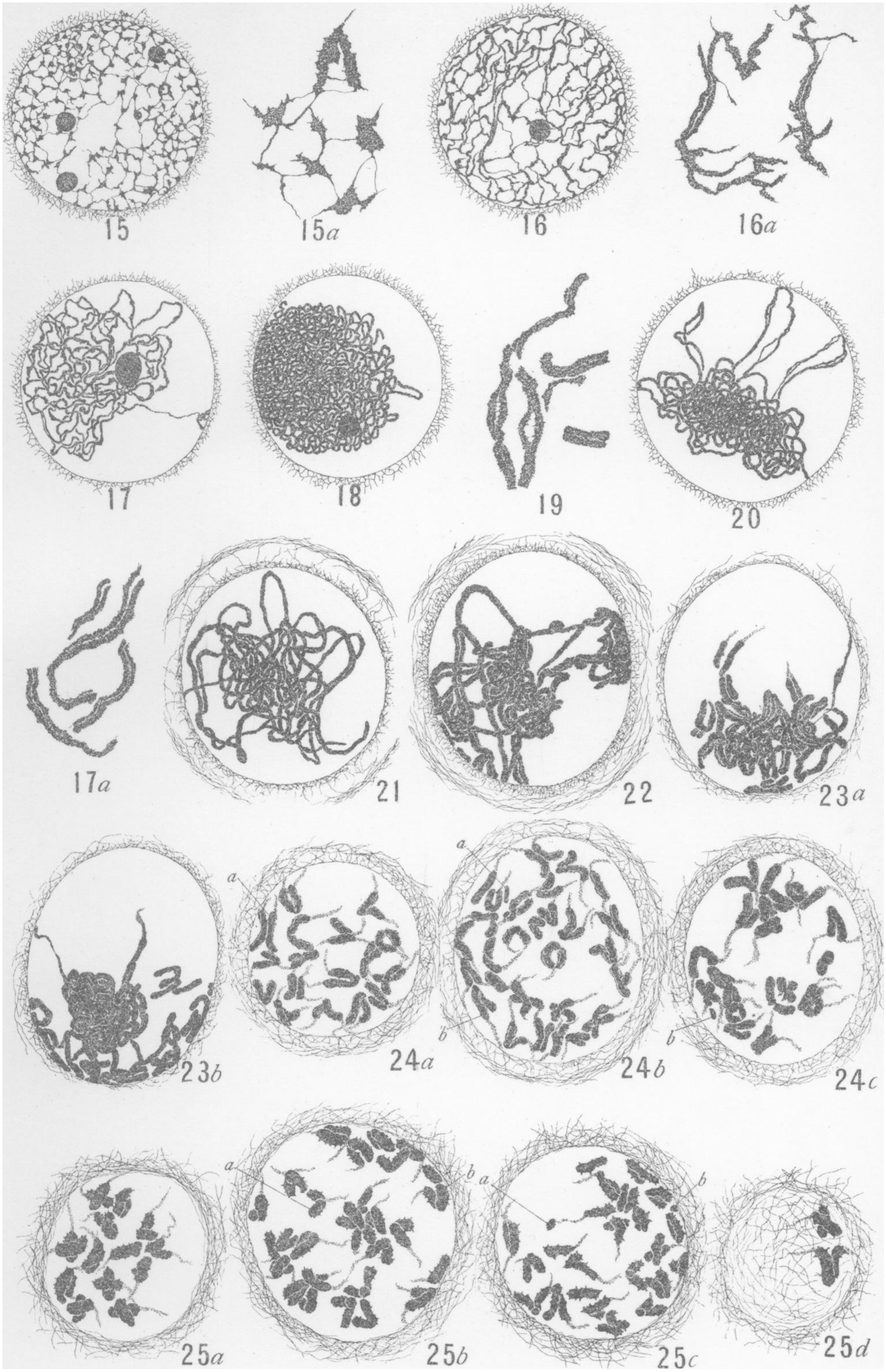
FIG. 60. Late telophase; membrane of the granddaughter nucleus formed; new fibers present between granddaughter nuclei of different hemispheres which were separated by granular zone in telophase of first division.

FIG. 61. Still later condition than stage shown in previous figure; cell plate completed through middle region of granular zone, thus dividing the two protoplasts separated in the first mitosis.



YAMANOUCHI on NEPHRODIUM

PLATE II.



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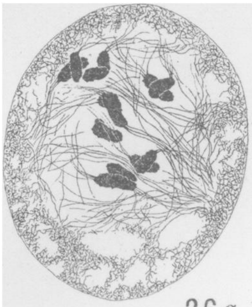
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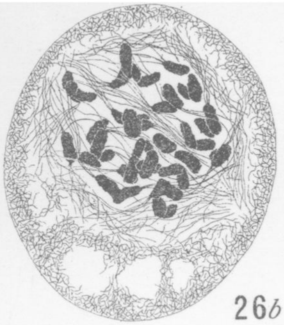
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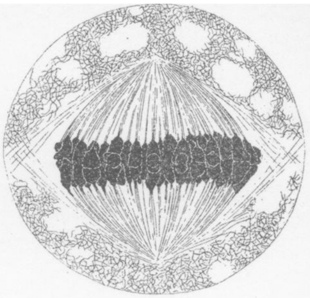
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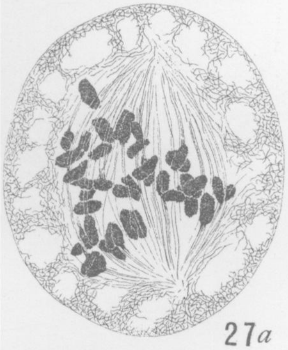
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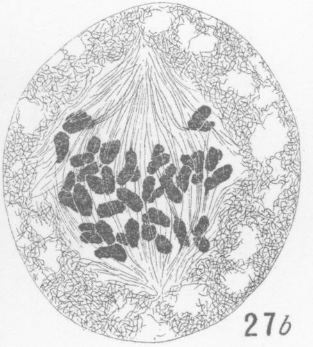
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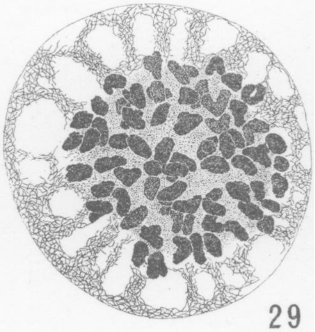
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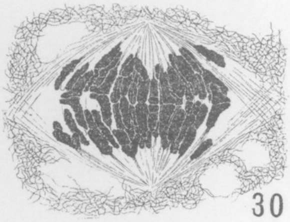
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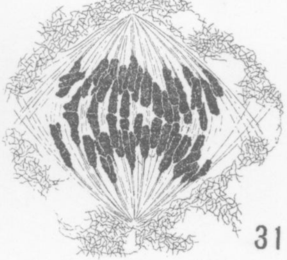
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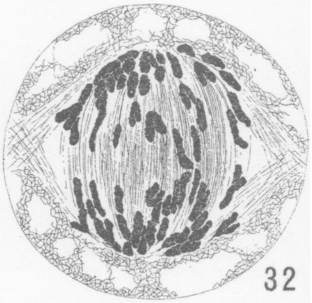
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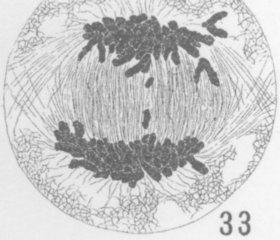
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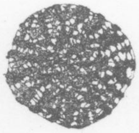
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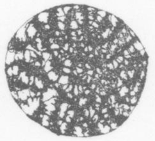
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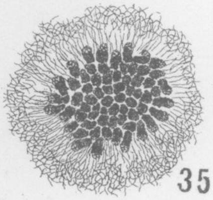
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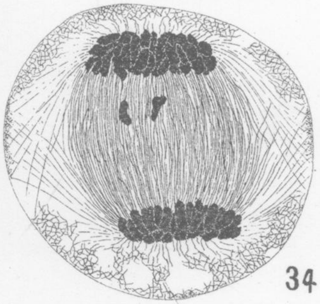
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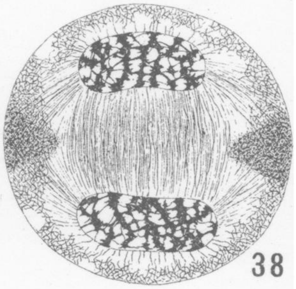
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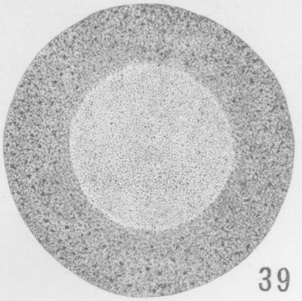
YAMANOUCHI on NEPHRODIUM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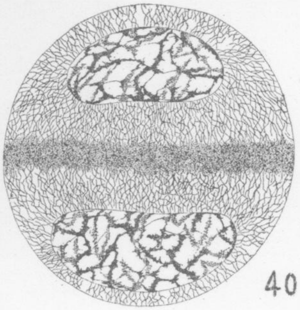
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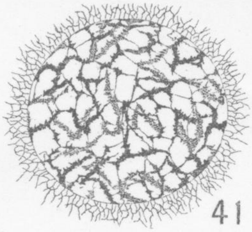
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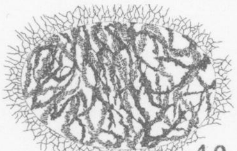
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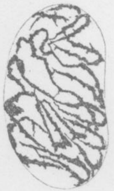
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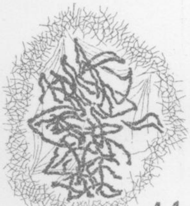
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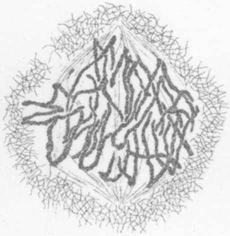
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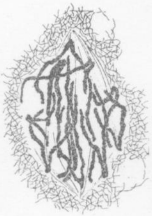
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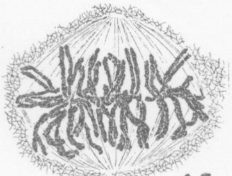
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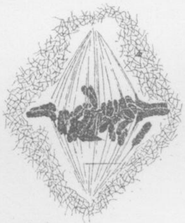
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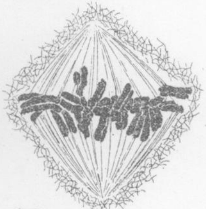
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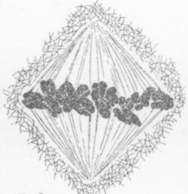
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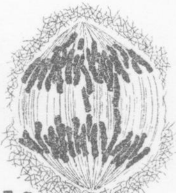
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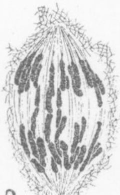
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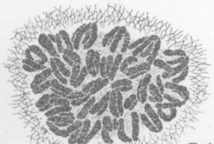
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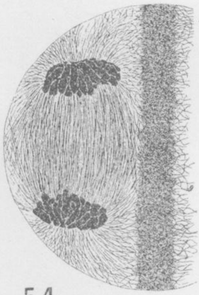
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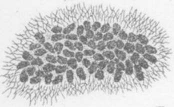
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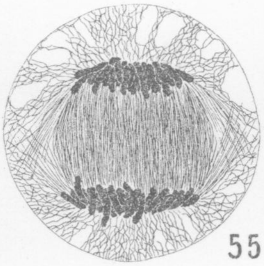
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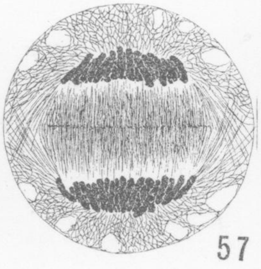
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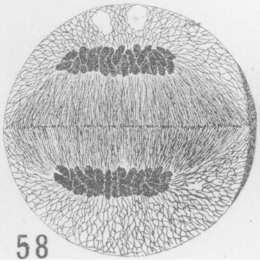
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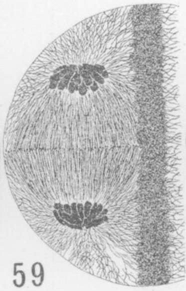
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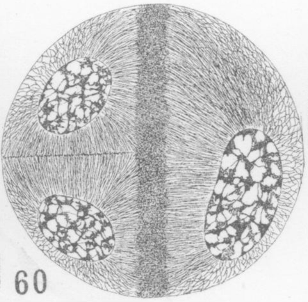
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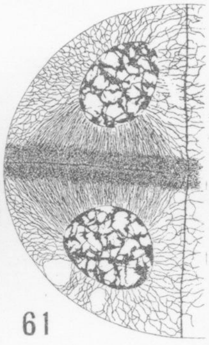
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